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Koolhaas, J.M.

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Hypothalamically Induced Intraspecific Aggressive Behaviour in the Rat

J.M. Koolhaas

Dept. of Zoology, State University of Groningen, Kerklaan 30, Haren, The Netherlands

Summary. The effects of electrical stimulation of the lateral hypothalamus (LH) of rats on intraspecific aggressive behaviour were studied. In order to investigate the specificity of the stimulation effects, each experimental animal was stimulated in a number of different social situations.

Stimulation of the LH in the presence of a subordinate male increased the amount of time spent on aggressive behaviour patterns and locomotion. In the presence of a dominant male, however, the stimulated animal never initiated a fight, whereas in the presence of an estrous female attack occurred in some rare occasions and sexual behaviour disappeared almost entirely. Stimulation of some sites also elicited mouse killing behaviour. Many of the electrodes that elicited intraspecific aggressive behaviour also supported intracranial self-stimulation.

It is concluded that 1. electrical stimulation of this area of the LH predominantly potentiates intraspecific aggressive behaviour, 2. that this behaviour becomes overt depends on the external situation, including the behaviour of the opponent.

Key words: Intraspecific aggression – Lateral hypothalamus – Electrical stimulation – Specificity – Rat

A number of experiments have demonstrated that in rats aggressive behaviour can be influenced by stimulating the brain electrically, by hormones, or by lesions. However, an integration of these data with ethological studies on aggressive behaviour of rats is seriously hampered, because both approaches often deal with different types of aggressive behaviour. For example in the brain-behaviour studies, predatory aggression (mouse killing) has been studied extensively by Karli (1971), whereas Brady and Nauta (1955) and Edwards and Adams (1974) analysed pain induced aggression, and Miczek et al. (1974) used food competition as a model for aggression. These types of aggressive behaviour are not identical in behavioural repertoire, sequential structure and

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physiological mechanisms (Moyer, 1971). On the other hand ethologists have dealt mainly with territorial aggression (Grant and McIntosh, 1963; Lehman and Adams, 1977) and hence it remains unclear to what extent the brain-behaviour experiments are helpful in understanding the central nervous regulation of territorial aggression of rats.

Although the main topic of their studies was stimulation bound mouse killing, two authors, Panksepp (1970) and Woodworth (1970) described intraspecific aggressive behaviour in male rats that were stimulated electrically in the lateral hypothalamic area. Panksepp (1970) found that only electrodes eliciting affective attack in the presence of a mouse, readily produced an attack towards an adult female rat. Although adult males seldom attack adult females entering their territory, there is at least a superficial similarity between this brain elicited behaviour and territorial aggression. Woodworth (1970) states that stimulation-bound attackers take into account the postures of the attacked conspecific males as do territorial males. Unfortunately, neither Panksepp nor Woodworth give any further description of the behaviour elicited. Since these two studies dealt only partially with intraspecific aggression during stimulation of the LH, the present paper analyses in more detail the behaviour effects of electrical stimulation of the same hypothalamic area of rats in a social setting.

In order to investigate the specificity of the behaviour effects of brain stimulation, it is necessary to study the range of behavioural responses elicited through one and the same electrode by testing it in a variety of experimental situations (Valenstein, 1970). In the present experiments stimulation-bound behaviour was recorded, while the males were in different social situations, that is, in the presence of a subordinate male, a dominant male, or an estrous female. Stimulation-bound mouse killing was also tested. Moreover, during testing, water and food were continuously available so that possible effects of the stimulation on feeding and drinking could also be determined. Also, initial stimulation experience may determine the type of stimulation bound behaviour. For that reason, in Experiment II, the order of social situations presented to the experimental animal was reversed.

Methods

Subjects

Male rats (strain WE_{zob}) of 300 gr and 3 months of age at the time of surgery were used as experimental animals. Each male was housed in a wooden observation cage with a foor space of 60 × 85 cm covered with wood shavings, and lived together with a sterilized female (by ligating the oviducts) to avoid the effects of long social isolation. During testing the female was removed from the cage. The experimental room was temperature controlled (22° C). A reversed day-night schedule of 12 hours light and 12 hours dark was used. All experiments were performed in the first 6 hours of the dark period.

Electrical Stimulation

Two stainless steel electrodes (∅ 0.2 mm), bare only at the cross section of the tip, were implanted unilaterally and behind each other into the LH under ether anaesthesia. One electrode was aimed at

the location Anterior: 6.2, Lateral: 1.5, and Ventral: –3.0 (De Groot, 1963). The other electrode was placed 0.5 mm behind the former. Both were angled at 20° to avoid damage to the stria terminalis. A stainless steel wire, wrapped around the screws on the skull served as a reference electrode.

Stimulation was monopolar with a biphasic sinewave current of 50 Hz.

Testing Procedure

All experiments were performed in the home cage of the experimental animal. After 1 week of recovery from the operation, current intensity was determined in the absence of a conspecific. Current intensity was chosen such that stimulation first elicited locomotory exploration. This intensity was used throughout all experiments and varied between individuals from 8 to 24 μA.

The behaviour of the stimulated animal was recorded in three different social situations:

a) Subordinate male tests: A male rat of the same strain, age and weight as the experimental animal was present in the home cage. Such an animal is almost certainly subordinate during encounters with the territory owner (the experimental animal);

b) Estrous female tests: An intact receptive female was used as a partner;

c) Dominant male tests: A male rat of 400 g of the aggressive strain Tryon Maze Dull S3 was present in the home cage.

In the subordinate- and dominant male tests the opponent was introduced 5 min before actually recording of the behaviour started. During this period the experimental animal could habituate to the newly introduced opponent. In the estrous female tests, this habituation period was terminated as soon as the first mounting was observed. Each behavioural test consisted of four different parts of equal duration. The first part of 150 sec, called the period before stimulation, is a control period during which no stimulation was given. In the following period of 5 min the stimulation was on and off for 10 sec alternately, giving again two periods of 150 sec each in total, called "during" and "interval", respectively. Finally a 150 sec period followed, called the period after stimulation.

Each animal was also tested for mouse killing behaviour and self-stimulation (ICSS).

1. Mouse killing test: A male mouse was introduced into the home cage of the experimental animal and left there for 10 min to determine possible spontaneous mouse killing. After this period, the animal was stimulated according to the usual schedule (10 sec on, 10 sec off) during 10 min. The latency of killing was measured.

2. ICSS test: These tests were carried out in the home cage of the experimental animal and in the absence of a conspecific. Each time the animal pressed the bar, it received an electrical brain stimulation of 0.4 sec of the same current intensity as used in the behavioural tests. If shaping was successful, the self-stimulation rate was determined in a test session of 30 min.

Sequence of Tests

Two groups of experimental animals were used. In the first group (Exp. I) the animals were initially tested exclusively in the presence of a subordinate male. The animals of the second group (Exp. II) were initially tested exclusively in the presence of an estrous female. After at least five of these behavioural tests, the animals were selected depending on their behaviour during stimulation. If the behaviour of the experimental animal was directed towards the opponent during stimulation further tests of the same type were made. If not, the electrode was considered as negative. After about eight behavioural tests of the same type, the positive electrodes of both groups of animals were tested in the other social situations. The self stimulation- and mouse killing tests were performed after completion of all behavioural tests. Each animal was tested twice a day and passed through the whole series of tests in about 4 weeks.

Recording of Behaviour

The behaviour of the experimental animal was continuously recorded by direct observation during all behavioural tests. For that purpose a set of behaviour patterns was selected in such a way that the animal is always engaged in one and only one of these patterns. Most of these patterns were derived from a study by Grant and MacIntosh (1963) and included the following acts and postures: attack,

upright, investigating, sideways, aggressive grooming, submissive, sniff anogenital region, mounting, genital grooming, sniffing, full aggressive posture, watching, feeding, moving towards and moving from. The occurrence of these behaviour patterns and the electrical stimulation was recorded on a 20-channel event recorder and on a magnetic tape, which could be analysed by a PDP-8 computer later on. In this study, the measure, used to analyse the behaviour, is the percentage of total time spent on each of the behaviour patterns recorded. The Wilcoxon Matched-pairs signed ranks test and the Mann-Whitney U-test were used for statistical analysis of the data (Siegel, 1957).

Histology

At the completion of all tests, the animals were anaesthetized and perfused with 0.9% saline followed by 10% formalin. Paraffin sections of 25 μm were cut. Every fourth section was preserved and stained with cresyl violet.

Results

Fourteen of the twenty-two operated animals showed a clear increase in social behaviour when stimulated in the presence of a conspecific. Eight of these were initially tested in the presence of a subordinate male (Exp. I) and six in the presence of an oestrous female (Exp. II). As there were no significant differences in the behaviour of the two groups towards the same conspecific stimulus, the data of all positive electrodes were pooled for each test situation. The negative electrodes all elicited an increase in sniffing and locomotion, but none of the social behaviour patterns was enhanced during stimulation.

Subordinate Male Tests

The important results are shown in Figure 1. The relative time spent on attack, upright posture, moving towards and moving from was significantly (Wilcoxon) increased by the electrical stimulation whereas the time spent on watching, self-grooming, investigating and sideways posture showed a significant decrease.

Qualitatively, the behavioural effects of the electrical stimulation can be described as follows: at the onset of the stimulation the animal usually starts some locomotory exploration, in the course of which it may approach the opponent. When the stimulated animal is close to the opponent, it will often start a fight. This fight is sometimes preceded by introductory patterns like investigating and sideways posture. After such a fight, the animal may start exploring the cage again, another fight may follow, or the opponent is kept in a submissive posture. The stimulated animal also adapts its behaviour to the actions of the opponent: for example, an opponent in submissive posture is not attacked. These behaviours stop almost immediately at the offset of the electrical stimulation. In the subsequent interval, watching and grooming are often performed.

A detailed analysis reveals that two types of aggressive responses can be distinguished on the basis of the repertory of social behavioural elements performed during stimulation (Koolhaas, in prep.). For the present purpose, the above general description is sufficient.

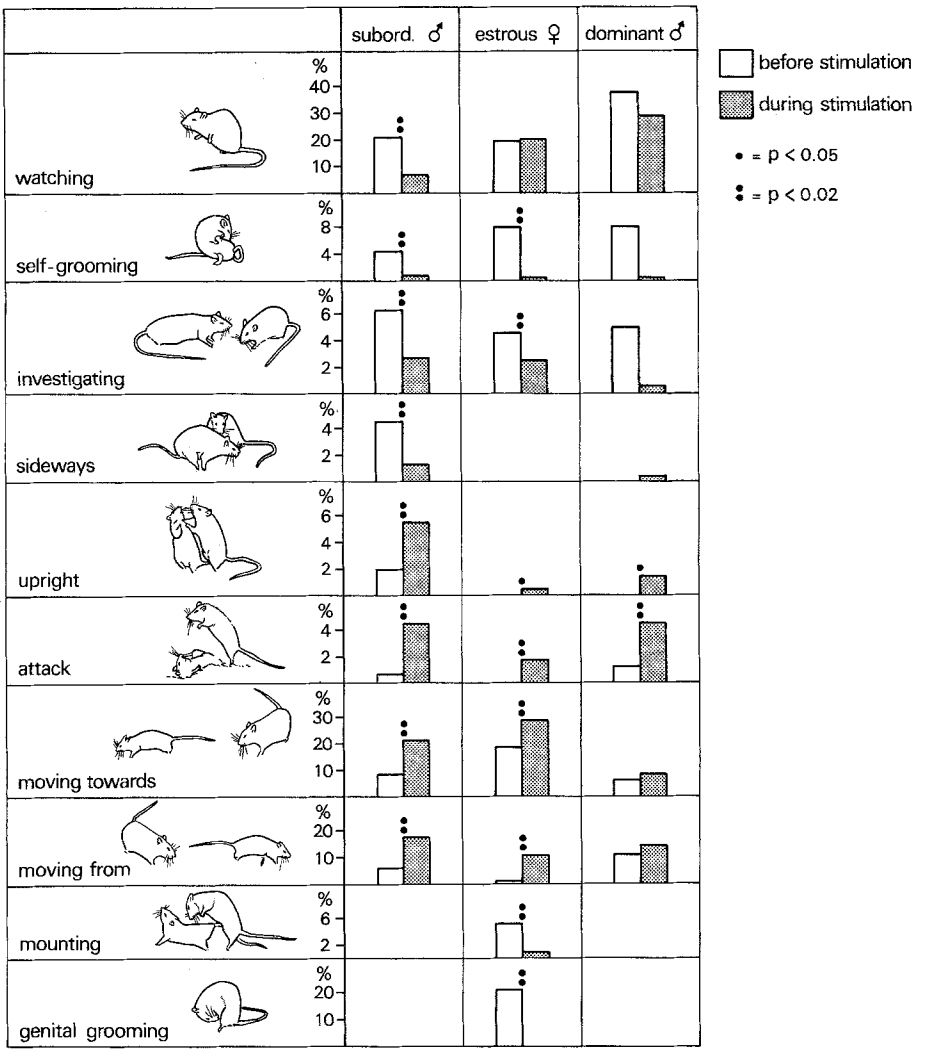

Fig. 1. Percentage durations of some behaviour patterns performed under normal and stimulation conditions in three different social situations. Total number of tests per situation: subord. male 119; estrous female 76; dominant male 28

Estrous Female Tests

Figure 1 also shows some of the behaviour changes during stimulation through all positive electrodes in the presence of an estrous female. Investigation, genital grooming, self-grooming and mounting, which form the major part of the behaviour in the control period, disappear almost entirely during stimulation ($p < 0.02$ Wilcoxon). Normally a male does not attack an estrous female, but during stimulation attack and upright posture are sometimes performed. These

fights are mostly very short and somewhat restrained. Attack is mostly observed when the female happens to be in front of the stimulated animal.

Some animals of Exp. II, which were tested initially in the presence of an estrous female, tend to perform mounting during stimulation. However, in all experimental animals, stimulation finally elicited attack. For the present purpose it is important to note that in this situation electrical stimulation still makes the animal behave aggressively, whereas sexual behaviour is strongly decreased in most cases.

Comparison of the behaviour during stimulation in this situation to that in the presence of a subordinate male (see Table 1) reveals that upright posture,

Table 1. Percentage durations of some behaviour patterns performed during stimulation in the presence of a subordinate male and in the presence of an estrous female

	subord. ♂	estrous ♀	significance (Mann-Whitney U test)
upright	5.32	0.54	$p = 0.02$
attack	4.43	1.88	$0.02 < p < 0.05$
sniffing	43.16	33.97	$p = 0.05$
moving from	13.80	11.27	$0.02 < p < 0.05$
moving towards	21.12	28.03	$0.02 < p < 0.05$

sniffing, moving from and attack occur significantly (Mann-Whitney) less in the estrous female tests, whereas significantly more moving towards is performed. This demonstrates that the behaviour during stimulation depends in part on the sex of the conspecific.

Dominant Male Tests

Behaviour in the presence of a heavy male S3 rat depends strongly on whether or not a dominance relationship between the experimental animal and the opponent has been established. Once the experimental animal has lost a fight (which occurs mostly in the habituation period), it will never initiate a fight again, neither in the control period, nor during stimulation. Instead of attacking during stimulation, the animal vacillates between moving towards and moving from, often interspersed with watching.

The significant (Wilcoxon) behavioural changes during stimulation through all positive electrodes in the presence of a dominant male are presented in Figure 1. Only upright posture and attack showed significant increase, whereas self-grooming decreases significantly. However, the increase of upright posture and attack are mainly caused by attack of the non-stimulated dominant partner which seems to be provoked by the increased restlessness of the stimulated

Table 2. Percentage durations of some behaviour patterns performed during stimulation in the presence of a subordinate male and in the presence of a dominant male

	subord. ♂	estrous ♀	significance (Mann-Whitney U test)
upright	5.32	1.95	$p = 0.02$
watching	5.90	29.18	$0.02 < p < 0.05$
moving towards	21.12	9.29	$0.02 < p < 0.05$

animal. This means that in this situation indirect and only minor changes in behaviour are evoked by the electrical stimulation.

Table 2 presents the significant (Mann-Whitney) differences between the behaviour in this situation and that in the presence of a subordinate male. Upright posture and moving towards are significantly less performed, whereas watching shows a significant increase. This demonstrates again that the behaviour during stimulation depends in part on the social situation in which it is tested.

Mouse Killing Behaviour

Eight of the fourteen positive electrodes also elicited mouse killing behaviour. With two electrodes the mouse was attacked without being killed. One experimental animal also performed spontaneous mouse killing and in this case stimulation reduced the latency of killing. Eating of the mouse after it was killed was never observed. The stimulation induced mouse killing was the same as described by Woodworth (1970).

Self-Stimulation

From eleven electrode placements ICSS was obtained. The self-stimulation rate varied between individuals from 200 to 1300 presses per hour. This variation appeared to be correlated with the type of aggressive behaviour elicited in the subordinate male situation (Koolhaas and Wiepkema, 1976).

Histology

The anatomical locations of the electrodes are presented in Figure 2. The positive electrodes were mainly located in the ventrolateral hypothalamus and range from anterior 5.8 to 6.8 (De Groot, 1964). The locations of three electrodes were lost in the histological procedure.

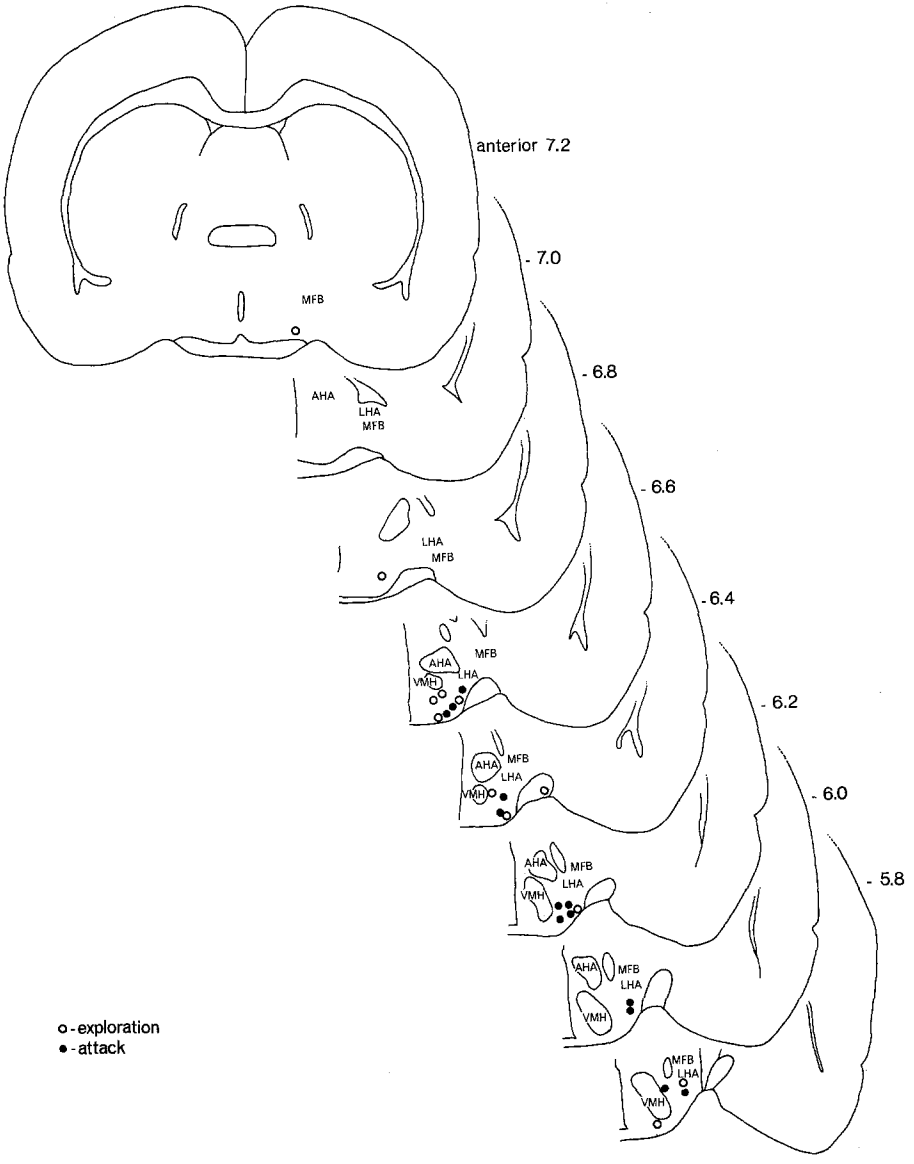

Fig. 2. Coronal sections (de Groot, 1963) showing the electrode tip locations

Discussion

All positive electrode placements presented in this study have in common that upright posture and attack were significantly increased during stimulation in all three social situations tested. This means that the stimulation induces aggressive behaviour patterns in some way. There are at least three obvious explanations for this:

a) The effect of the stimulation is a painful sensation which in turn triggers aggressive behaviour (Plotnik, 1974);

b) The effect of the stimulation is primarily an increase in locomotory, activity which consequently enhances the chance of meeting the opponent;

c) The electrode directly activates a neural structure subserving aggression behaviour.

The first possibility is unlikely because most of the experimental animals also perform self-stimulation behaviour at the same current intensity as used in the behavioural tests. Some preliminary experiments showed that also self-stimulation could be obtained with stimulus durations of 10 sec or even more.

Moreover, escape or screaming was never observed during brain stimulation. The second possibility can also by ruled out for two reasons. Firstly, the negative electrode placements all elicited an increase in locomotion, but failed to produce aggressive behaviour. Secondly, it does not explain the occurrence of aggressive behaviour in the estrous female tests. On the contrary, sexual behaviour should have occurred.

It might be concluded therefore, that the increase in aggressive behaviour is the result of a direct activation of a neural structure subserving at least intraspecific aggressive behaviour. However, before accepting such a view it is necessary to find out whether the electrode does not activate other behaviour patterns as well. In this context, the results of the estrous female tests are relevant, in that finally all experimental animals performed stimulation induced aggressive behaviour in this situation. However, in Exp. II, in which the animals were initially tested in the presence of an estrous female, mounting occurred during stimulation. Some animals mounted the female in every test, others switched from mounting to attack in the course of the tests. Here we meet the same problen of the interpretation of multiple behaviour patterns elicited from one electrode, as has been extensively discussed by Valenstein (1970a, b, c), Roberts (1969), and Wise (1968, 1974) for feeding, gnawing, and drinking. Since the final result in the estrous female situation is stimulation induced aggression in all cases, it can be concluded that the dominant effect of electrical stimulation in this area of the LH is aggression. Other behaviours like feeding and drinking were never observed during stimulation, but the contribution of the stimulation to the occurrence of mounting remains unclear and will be discussed elsewhere (Koolhaas, in prep.).

Assuming that normally this particular area of the LH plays a role in aggressive behaviour, we may ask how this brain structure contributes to such behaviour. This study has shown that the animal does not fight each time it is stimulated, but is able to adapt its behaviour to the external situation. This suggests that the LH does not exert a final executive role: the stimulation just enhances the probability of occurrence of aggressive behaviour. Whether or not this behaviour will be performed seems to depend on external and possibly also internal variables. This is a quite common phenomenon in hypothalamically induced behaviour. For instance in Rhesus monkeys, the social status of both the experimental animal and the opponent is an important determining factor in aggressive behaviour, both during stimulation and under normal circumstances (Alexander and Perachio, 1973; Delgado, 1967). Also in the cat it has been

demonstrated that stimulation-induced interspecific attack is under sensory control (Flynn, 1969; MacDonnel and Flynn, 1966). In the rat this phenomenon has been shown in drinking (Phillips and Mogenson, 1968) and feeding behaviour (Prop-van den Berg et al., 1977).

Therefore, an important line for further research will be to find out if also internal variables are taken into account in gating the output of the lateral hypothalamic structure subserving intraspecific aggressive behaviour.

Some animals also showed stimulation-induced mouse killing. Since not all electrodes that produced intraspecific aggressive behaviour elicited muricide, at least under the conditions of the present experiments, the conclusion by Moyer (1971) is strengthened that intra- and interspecific aggressive behaviour are based on partially different physiological mechanisms.

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